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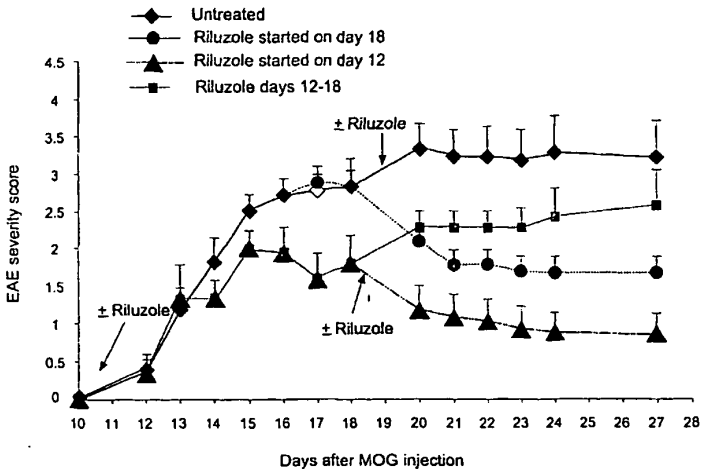
(54) Title: PHARMACEUTICAL COMPOSITION FOR THE TREATMENT OF MULTIPLE SCLEROSIS



(57) Abstract: Riluzole, a drug that inhibits glutamethargic release, is shown to be effective in the prevention and treatment of multiple sclerosis (MS). The effect of Riluzole is shown in an animal model of MS, an experimental autoimmune encepthalomyelitis (EAE) model produced by injection of myelin oligondendrocyte glycoprotein (MOG) to animals. Administration of Riluzole to such animals before they develop the MS-related symptoms markedly reduced the incidence and clinical severity of the disease in such animals. Moreover, treatment of such animals after the appearance of severe MS-related symptoms, also markedly slowed down the progression of the disease and improved the clinical manifestations.

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PHARMACEUTICAL COMPOSITION FOR THE TREATMENT OF MULTIPLE SCLEROSIS

FIELD OF THE INVENTION

This invention concerns pharmaceutical compositions for the treatment of multiple sclerosis (MS).

BACKGROUND OF THE INVENTION

Multiple Sclerosis (MS) is a disorder of the central nervous system, involving decreased nerve function associated with the formation of scars on the myelin covering of nerve cells. This disease affects approximately 1 out of 1,600 people, with women being affected 60% of the time. The disorder most commonly begins between the ages of 20 to 40, and is one of the major causes of disability in adults under age 65.

Multiple sclerosis involves repeated episodes of inflammation of nervous tissue in any area of the central nervous system, including the brain and spinal cord. The location of the inflammation varies from one person to another and from episode to episode. The inflammation destroys the myelin sheath covering the nerve cells in that area, which causes the formation of multiple areas of scar tissue (sclerosis) along the covering of the nerve cells. Sclerosis slows or blocks the transmission of nerve impulses in that area, resulting in the development of the symptoms of MS.

Symptoms vary considerably, since the location and extent of each attack varies. There is usually a stepwise progression of the disorder. At the initial stages ("*relapsing-remitting*" stage) the episodes of onset of symptoms last days, weeks or months, alternating with times of reduced or no symptoms (remission) and periods of recurrence (relapse). During relapse there is an appearance of a new symptom, the reappearance of a previous symptom or the worsening of an existing symptom. At more advance stages (termed: "*chronic-progressive*" stage which may be either

primary or secondary), there is a progressive deterioration of nerve function which is probably caused by the irreversible destruction of nerve axons.

The exact cause of the inflammation associated with MS is unknown. Several geographic studies indicate that there may be an environmental factor involved with MS. There seems to be a familial tendency toward the disorder, with a higher incidence in certain family groups that in the general population indicating a possible genetic involvement. An increase in the number of immune cells in the body of a person with MS indicates that there may be a type of immune response that triggers the disorder.

The most frequent theories about the cause of multiple sclerosis include: infection by a virus-type organism; an abnormality of the genes responsible for control of the immune system; or a combination of both factors.

There is no known cure for multiple sclerosis and treatment is aimed at controlling systems and maintaining functions to give the patient a maximum quality of life.

Medications vary depending on the symptoms that occur. Baclofen, dantroene, diazepam or other anti-spasmodic medications are used to reduce muscle spasticity. Cholinergic medications may be helpful to reduce urinary problems. Antidepressant medications may be helpful for mood or behavior symptoms. Amantadine may be given for fatigue.

Corticosteroids or ACTH are frequently used to suppress the inflammation in an attempt to reduce the duration of an attack. Medications that suppress the immune are also often used. Recently it has been found that Interferon may also be helpful for some people.

Recent studies (Pitt *et al.*, *Nature* Medicine, 6(1), 67-70, 2000; Smith *et al.*, *Nature Medicine*, 6(11) 62-66, 2000) have indicated that autoimmune Encephalomyelitis, which is a model for MS, was ameliorated by AMPA/kainate which is an antagonist of the glutamate receptor.

Riluzole (2-amino-6-trifluoromethoxy benzothiazole) has recently emerged as a pharmacological agent potentially useful to slow down the evolution of

neurodegenerative diseases, such as amyotrophic lateral sclerosis. (Ben Simon *et al., New Engl. J. Med.* 330:585-91 (1994)). In addition, this molecule has been shown to display anticonvulsant, anti-ischemic, and neuroprotective properties under various experimental conditions. A clear understanding of the site and mechanism of action of this molecule is still lacking.

SUMMARY OF THE INVENTION

The present invention is based on the surprising findings that in an animal model of multiple sclerosis (MS) an experimental autoimmune encephalomyelitis (EAE) model produced by an injection of myelin oligodendrocyte glycoprotein (MOG), animals treated by the administration of Riluzole before the appearance of clinical MS-related symptoms developed virtually no such clinical symptoms ,and appeared essentially normal in all aspects tested. Moreover, treatment of such animals with Riluzole after the appearance of MS-related symptoms completely arrested the progression of the disease. Furthermore, some improvement in the animal's condition had been shown.

The present invention provides, by one of its aspects, a pharmaceutical composition for the treatment of multiple sclerosis, comprising a pharmaceutically acceptable carrier, and, as an active ingredient, Riluzole.

The term *"treatment"* in the context of the invention refers to any one of the following: amelioration of some of the undesired symptoms of multiple sclerosis; the prevention of the manifestation of such symptoms before they occur; slowing down or completely preventing the progression of the disease (as may be evident by longer periods between reoccurrence episodes, slowing down or prevention of the deterioration of symptoms etc.); enhancing the onset of the remission period; slowing down the irreversible damage caused in the progressive-chronic stage of the disease (both in the primary and secondary stages); delaying the onset of said progressive stage, or a combination of two or more of the above.

Thus, it is clear that the pharmaceutical compositions of the invention are suitable to be administered to patients in all stages of the disease of multiple

sclerosis, including the initial relapsing-remitting stages, both during remission periods (to prevent or delay reoccurrence) or reoccurrence conditions (to expedite remission and delay the onset of the progressive stage), as well as in chronic-progressive stages.

The term "*multiple sclerosis*" refers also to any other autoimmune disease manifested by demylination of the central nervous system's neurons. The first symptoms which appear at the onset of MS will be referred to herein at times as *"MS-related symptoms"*. The symptoms of MS in EAE –induced animals (animal model of MS) are typically weakness and malfunction in the animal's tail, followed by weakness of its rear feet and finally weakness in its front feet. In humans, such first MS-related symptoms may typically be double vision, facial numbness, facial weakness, vertigo, nausea, vomiting ataxia, weakness of the arms, etc.

The term "*Riluzole*" refers to (2-amino-6-trifluormethoxy-benzothiazole) and chemical modifications (such as addition, replacement or deletion of a substituent) of the above. Those modifications which fall under the scope of the invention are those that feature the therapeutically effect of Riluzole (for example in the model specified in the "detailed description" part) at concentrations which are the same or lower than those of Riluzole.

Typically, the pharmaceutical composition of the invention may be administered to patients in any manner known in the art, such as parenteral administration, (intravenously, or intraparenteral), by injection or by oral administration, oral administration being preferred.

The pharmaceutically acceptable carrier should be formulated in accordance with the intended mode of administration of the pharmaceutical composition, as evident to any person versed in the art. For oral administration, the Riluzole may for example be formulated as a coated tablet comprising Riluzole as an active ingredient together with a pharmaceutically acceptable excipient such as, for example, calcium phosphate, microcrystalline cellulose, silica, magnesium stearate, polyethylene glycol, etc.

The dosage of the Riluzole in the pharmaceutical composition should be determined empirically for each individual, and should depend on various factors, such as the patients' age and weight, the stage of the multiple sclerosis (remitting-relapsing; chronic-progressive), the severity of the symptoms of each stage etc. For example only, the dosage of Riluzole which may be typically administered to an MS patient taken orally with the drug may be in the range of between about 50 mg to about 200 mg per day, preferably about 100 mg per day administered in two doses of 50 mg every 12 hours.

If desired, the pharmaceutical composition of the invention may further comprise other therapeutically effective agents which are known to be beneficial for the treatment of multiple sclerosis.

The present invention further concerns use of Riluzole for the preparation of a medicament for the treatment of multiple sclerosis.

By another aspect, the present invention concerns a method for treatment of multiple sclerosis, comprising: administering to a subject in need of such treatment, a therapeutically effective amount of Riluzole. Preferably the administration should be by oral administration.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 shows the mean clinical score of an EAE model of MS (mice injected with MOG) as a function of time after injection, for untreated mice (empty squares) and mice treated with Riluzole (full circles).

Fig. 2 shows the EAE clinical score model of MS (mice injected with MOG) as a function of time after injection, in mice treated with Riluzole (circles) and in untreated mice (squares).

Fig. 3 shows the EAE severity score model of MS (mice injected with MOG) as a function of time after injection, in mice treated with Riluzole at various times (circles, squares and triangles) and in untreated mice (rhombus squares).

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

Example 1: Effect of Riluzole in an MS model.

Experimental Autoimmune Encephalomyelitis (EAE) induction:

EAE was induced in C3H.SW female mice (6 weeks old, Harlen, Rehovot, Israel) by immunization with the peptide encompassing amino acids 35-55 of rat myelin oligodendrocyte glycoprotein (MOG). Synthesis was carried out by the Weizmann Institute Synthesis Unit, using a solid-phase technique on a peptide synthesizer (Applied Biosystems Inc., Foster City, CA City).

Mice were injected subcutaneously at one site in the flank with a 200μl emulsion containing 75μg MOG peptide in complete Freund's adjuvant (CFA) and 200μg Mycobacterium tuberculosis (Sigma Israel).

Treatment:

Mice (n=10) were injected i.p. with Riluzole 10mg/kg twice a day for 7 days before MOG injection and for additional 53 days after injection. Control mice (n=10) were injected twice a day with 200μl of saline for the same period.

Clinical score assessment:

0 = no clinical symptoms;

1 = loss of tail tonicity;

2 = partial hind limb paralysis;

3 = complete hind limb paralysis;

4 = paralysis of four limbs;

5 = total paralysis;

6 = death.

Results:

The results are shown in Fig. 1. Following the encephalitogenic challenge, mice were observed daily and clinical manifestations of EAE were scored. Four weeks after the encephalitogenic challenge, the saline injected mice (5/10) developed severe EAE characterized by complete hind limb paralysis (mean total score of 1.7+ 0.2 SE) starting on day 36. In contrast, the Riluzole-treated mice were significantly resistant to MOG-induced EAE. Nine out of the ten immunized Riluzole-treated mice remained disease free with only one mouse demonstrated clinical signs only on day 54 ($p=0.00002$ using X2 values) with mean of total score of 0.1+0.1 SE, ($p=0.015$ using Student-t-test).

Thus, Riluzole treatment markedly reduced both the incidence and clinical severity of the disease.

Example 2: Effect of Riluzole administered more than a week after the appearance of MS symptoms

EAE was induced in C3H.SW mice by injection of MOG as described in Example 1 above. The effect of administration of Riluzole on the progression of MS **after** the appearance of the first MS-related symptoms in the mice was tested.

Treatment:

Mice received MOG as described in Example 1 above and upon appearance of the first MS-related symptoms were divided into the following groups (n=10 in each group):

1. Mice receiving Riluzole 14 days after the appearance of the symptoms;
2. Mice which did not receive Riluzole (Control).

Riluzole was administered to the mice at a dose of 10mg/kg twice a day until the end of the experiment

The EAE score was calculated as described in Example 1 above.

Results:

As seen in Fig. 2, the first MS related symptoms were detected seven days after the injection of MOG. Mice were treated with Riluzole at day 14 and as seen in the Fig., at day 15 there is a cessation in the development of MS in the treated mice with no alteration of the clinical score in these mice from this time on.

Example 3: Effect of Riluzole administered at various times, more than a week after the appearance of MS symptoms

EAE was induced in C3H.SW mice by injection of MOG as described in Example 1 above. The effect of administration of Riluzole on the progression of MS after the appearance of the first MS-related symptoms in the mice was tested.

Treatment:

Mice received MOG as described in Example 1 above and upon appearance of the first MS-related symptoms were divided into the following groups (n=10 in each group):

1. Mice receiving Riluzole upon the appearance of the symptoms, from day 12 to day 18;
2. Mice receiving Riluzole after the severe appearance of the symptoms (score=3.2 in an EAE model), from day 18 to day 28;
3. Mice which received Riluzole after the appearance of the symptoms on day 12, but stopped receiving Riluzole on day 18; and
4. Mice which did not receive Riluzole (Control).

Riluzole was administered to the mice at a dose of 10mg/kg twice a day until the end of the experiment (day 28).

The EAE score was calculated as described in example 1 above.

Results:

As seen in Fig. 3, sixteen days after the appearance of the first MS-related symptoms, mice that had been treated with Riluzole showed the lowest scores of EAE as compared to the untreated mice (0.9 + 0.26 vs. 3.25 + 0.48, $p<0.0004$). Moreover, mice that started the Riluzole treatment in the late stage of the disease (on day 18) demonstrate significant improvement in their clinical score. Furthermore, Riluzole-treated mice that stopped the treatment on day 18 showed increase in the clinical score.

Thus, the results of these two experiments show that, in addition to the effect of Riluzole administered to EAE-induced animals before they had developed first symptoms of the disease (Example 1), Riluzole treatment of EAE-induced animals shortly after the animals had developed first symptoms of the disease also markedly slowed down the progression of EAE and even improved the clinical manifestation.

CLAIMS:

1. A pharmaceutical composition for the treatment of multiple sclerosis (MS) comprising a pharmaceutically acceptable carrier and as an active ingredient, Riluzole.

2. A pharmaceutical composition for the treatment of autoimmune-demylination of nerves comprising a pharmaceutically acceptable carrier and, as an active ingredient, Riluzole.

3. A pharmaceutical composition according to claims 1 or 2 for oral administration.

4. Use of Riluzole for the preparation of a medicament for the treatment of multiple sclerosis.

5. A method for the treatment of multiple sclerosis comprising administering to a subject in need of such treatment, a therapeutically effective amount of Riluzole.

6. A method according to claim 5, wherein the Riluzole is administered orally.

7. A pharmaceutical composition according to claims 1 or 2, wherein said composition is administered after the appearance of MS.-related symptoms.

8. A pharmaceutical composition according to claims 1 or 2, wherein the treatment is administered before the appearance of MS-related symptoms.

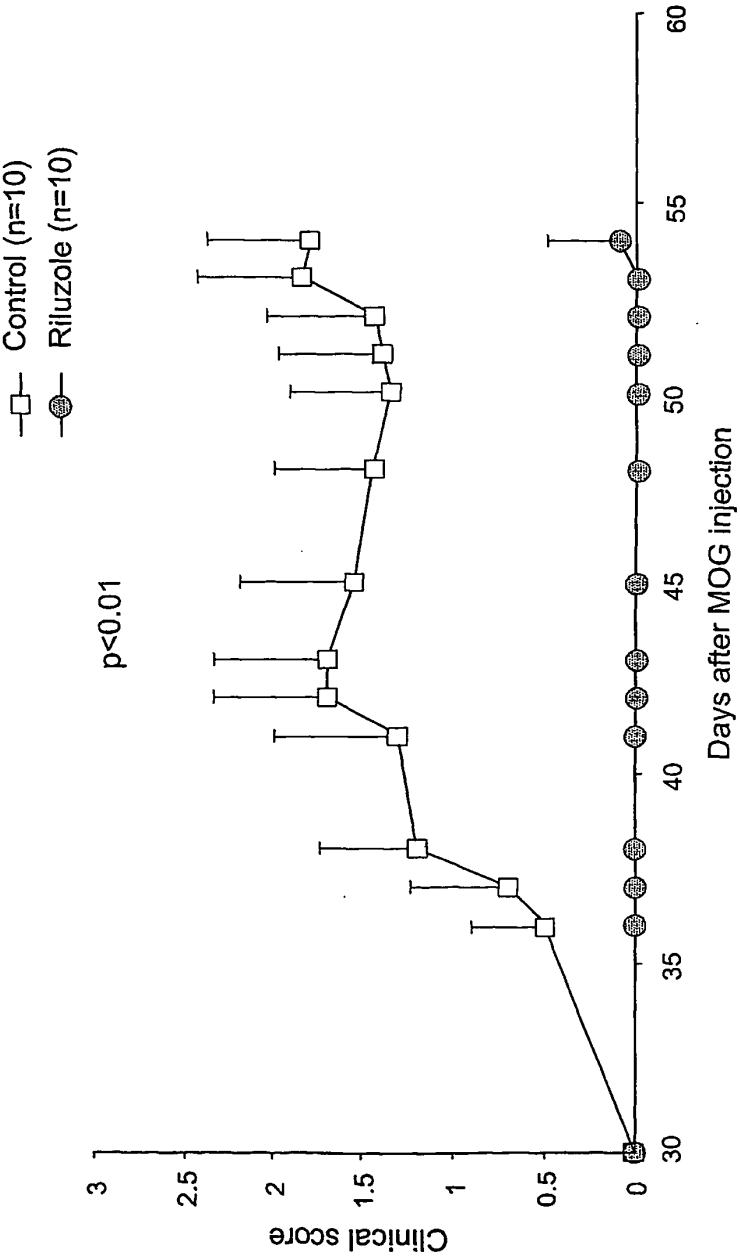
Control (n=10)
Riluzole (n=10)
p<0.01
Clinical score
Days after MOG injection
0
0.5
1
1.5
2
2.5
3
30
35
40
45
50
55
60

FIG. 1

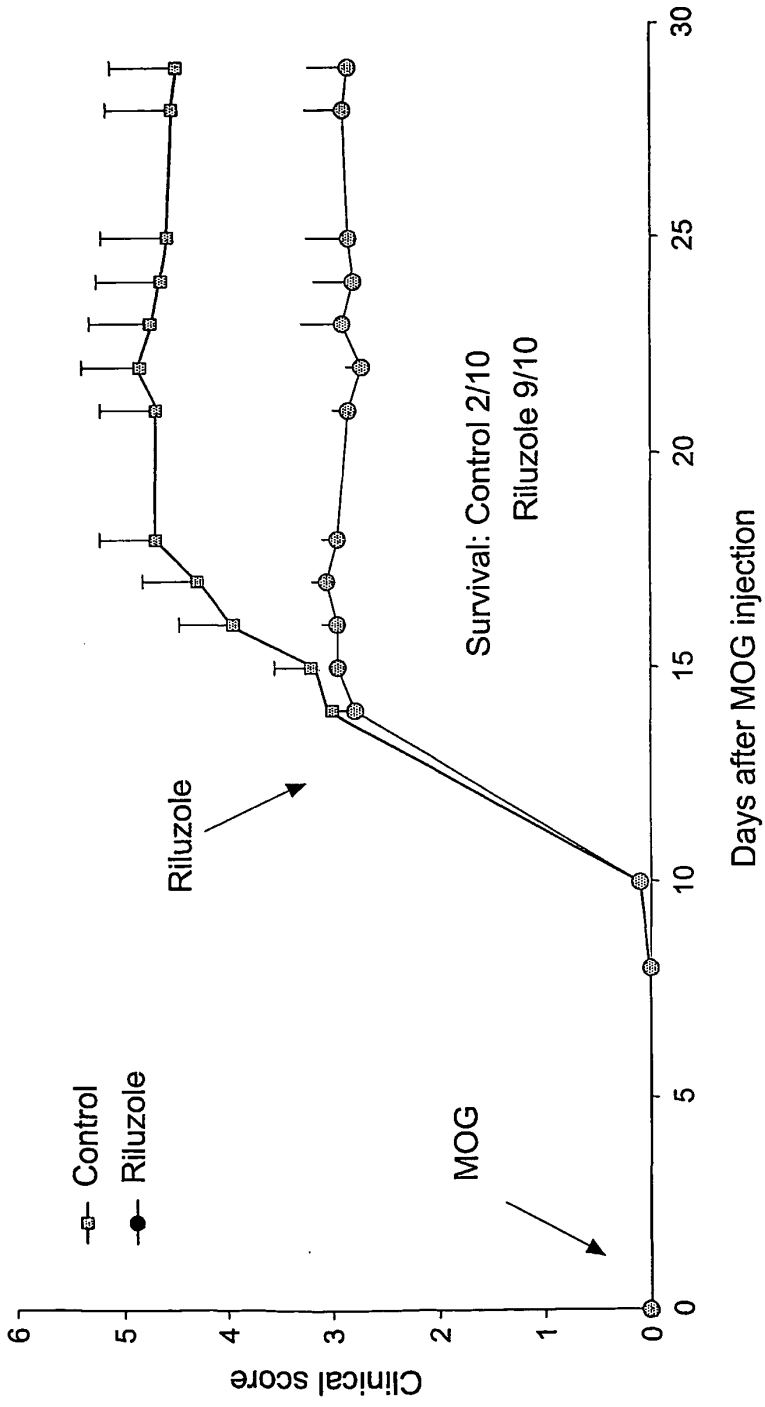
Clinical score
Days after MOG injection
Control
Riluzole
MOG
Riluzole
Survival: Control 2/10
Riluzole 9/10
0
1
2
3
4
5
6
0
5
10
15
20
25
30

FIG. 2

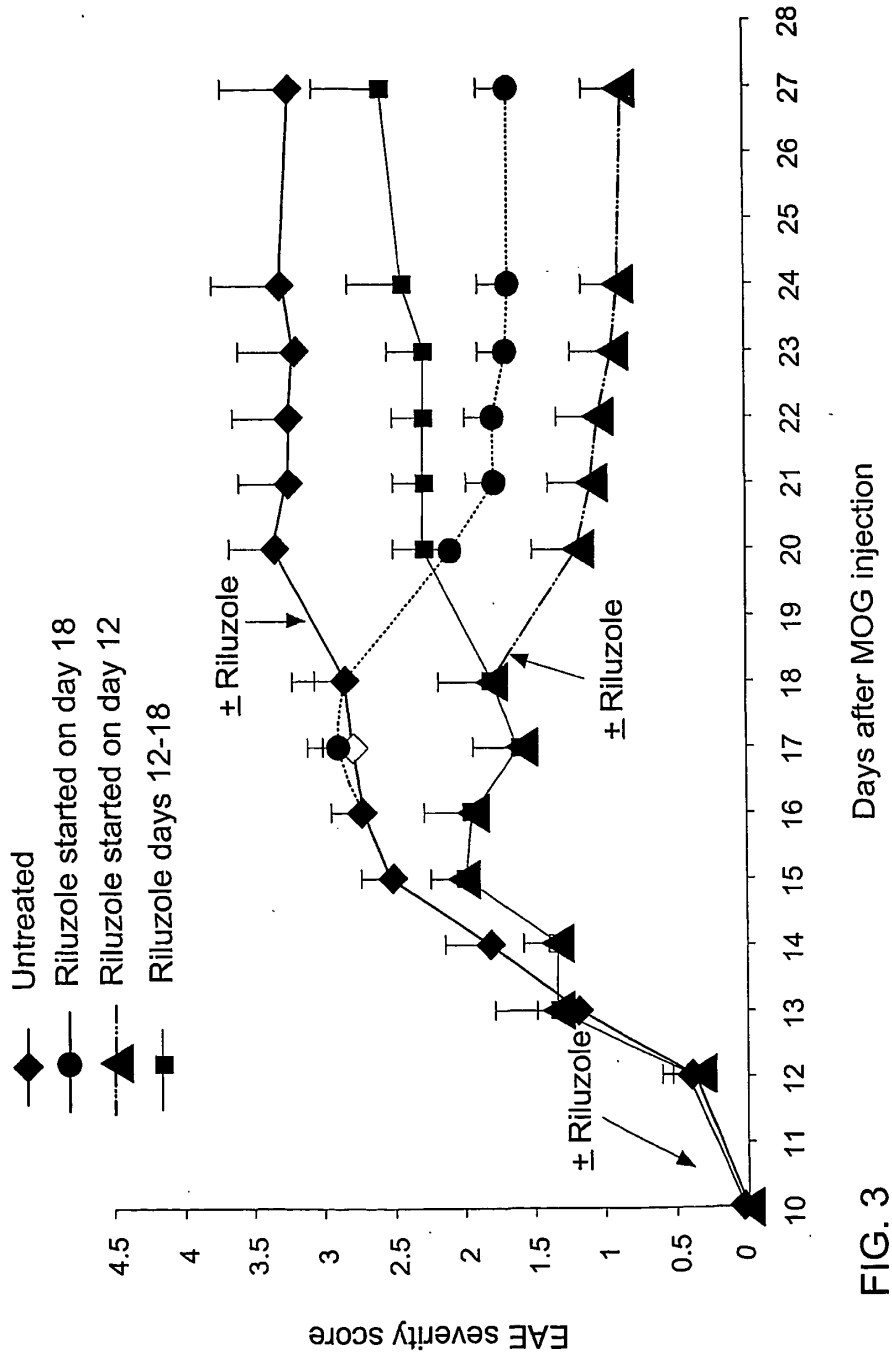
Untreated
Riluzole started on day 18
Riluzole started on day 12
Riluzole days 12-18
± Riluzole
± Riluzole
± Riluzole
EAE severity score
Days after MOG injection
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
10
11
12
13
14
15
16
17
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FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 01/00534

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/428 A61P25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, MEDLINE, BIOSIS, SCISEARCH, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 00 74676 A (POLMAN CHRIS ;VERENIGING VOOR CHRISTELIJK WE (NL); BIOGEN INC (US)) 14 December 2000 (2000-12-14) the whole document	1-8
P,X	FERNANDEZ O.: "'The rational basis of the newer treatments used in multiple sclerosis !. BASE RACIONAL PARA LOS NUEVOS TRATAMIENTOS EN LA ESCLEROSIS MULTIPLE." REVISTA DE NEUROLOGIA, (16 JUN 2000) 30/12 (1257-1264). , XP001041153 abstract; table 1	1-8

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
19 November 2001	26/11/2001

Name and mailing address of the ISA	Authorized officer
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International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KALKERS N F: "A pilot study of riluzole in primary progressive multiple sclerosis;effect on spinal cord atrophy on MRI" AVAILABLE FROM INTERNET, 6 June 1999 (1999-06-06), XP002122164 the whole document	1-8
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Information on patent family members

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